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Application No.	<u>09/126816</u>	Prepared by	<u>TW</u>	Tracking Number	<u>✓942818</u>
Examiner-GAU	<u>NICKOL-16Y2</u>	Date	<u>9-7-04</u>	Week Date	<u>4-26-04</u>
		No. of queries	<u>1</u>		<u>JFW</u>

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a. Serial No.	f. Foreign Priority	k. Print Claim(s)	p. PTO-1449
b. Applicant(s)	g. Disclaimer	l. Print Fig.	q. PTOL-85b
c. Continuing Data	h. Microfiche Appendix	m. Searched Column	r. Abstract
d. PCT	i. Title	n. PTO-270/328	s. Sheets/Figs
e. Domestic Priority	j. Claims Allowed	o. PTO-892	t. Other

SPECIFICATION	MESSAGE
a. Page Missing	
b. Text Continuity	
c. Holes through Data	
d. Other Missing Text	
e. Illegible Text	
f. Duplicate Text	
g. Brief Description	
h. Sequence Listing	
i. Appendix	
j. Amendments	
<input checked="" type="checkbox"/> k. Other	<p>There is a discrepancy between the drawings and specification in reference to Figure 4. Drawings show Figure Y-1 (A + D) and Figure Y-2 (B+C). Drawings dated 7-31-98 Specification dated 7-5-00 changes line 11, 12, 13, and 14 reads YA(i) to YD, YA(ii), YB(i), YE(iii) YD, etc</p>
CLAIMS	<p>Specification dated 5-21-02 for line 20 for Figures YA - YE referenced Please verify</p>
a. Claim(s) Missing	
b. Improper Dependency	
c. Duplicate Numbers	
d. Incorrect Numbering	
e. Index Disagrees	
f. Punctuation	
g. Amendments	
h. Bracketing	
i. Missing Text	
j. Duplicate Text	
k. Other	<p>Called Attorney - and discrepancy has been fixed via the attached supplemental examiner's amendment.</p>
	<p>Initials TW</p>
	<p>Initials GJW</p>

EXAMINER'S AMENDMENT

An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

Authorization for this examiner's amendment was given in a telephone interview with Thomas A. Cawley Jr., Ph.D. on November 16, 2004.

The application has been amended as follows:

On page 20, line 4, please replace the paragraph with the following rewritten paragraph:

To identify the acceptor amino acid glucosylated by LT, H-Ras protein as modified by LT in the presence of UDP-[¹⁴C]Glc, electrophoresed on SDS-PAGE, digested with trypsin and the resulting peptides were separated, as described in sections 1 and 2. As shown in Fig. 4, Panel 4-1A, 47 fractions were obtained. The radioactivity was exclusively associated with fractions 39 and 40. As shown in Fig. 4, Panels 4-2B and 4-2C, repurification of fraction 39 or 40 gave rise to a major peptide (D for 39 and E for 40) containing the radioactivity and several other small peptides. Peptides D and E were microsequenced and gave exactly the same amino-acid sequence. Each cycle of Edman degradation was collected and counted for radioactivity. The following unambiguous sequence was found for these peptides SALTIQLIQNHFVDEYDPTIEDSYR (SEQ ID NO.: 5). Cycle 19 corresponding to a threonine gave a very small signal. The small amount of threonine detected in position 19 may be the consequence of the LT catalyzed glucosylation of most of Ras molecules present in the reaction. Decrease or absence of threonine 37 Rho A in automated

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amino-acid sequencing, after glycosylation by toxin A or B, has been already reported (Just, I. et al., Nature 375 (1995) 500-503; Just, I., et al. J. Biol. Chem..270 (1995) 13932-13936). The amino-acid sequence found for both peptides D and E corresponds exactly to a sequence found in the H-Ras protein between amino-acids 17 to 41 (Barbacid, M., Ann. Rev. Biochem. 56 (1987) 779-827). Radioactivity was associated first with cycle 19 and decreased thereafter. The rise in radioactivity at cycle 19 establishes threonine 35 (of the H-ras molecule) as the unique amino-acid glycosylated by LT.

On page 14, line 11, please replace the paragraph with the following rewritten paragraph:

Fig. 4: Localization of the LT catalyzed (¹⁴C) glycosylation in H-Ras by microsequencing: Panel 4-1A; Separation by HPLC of the peptides generated by trypsin and radioactivity of each fraction (on 15 ml aliquot). Panels 4-2B and 4-2C; Purification by HPLC of fractions 39 and 40. Radioactivity associated with each peptide was counted on 50 ml aliquots. Panel 4-1D; Radioactivity associated with each Edman degradation cycle (each Edman cycle of peptide E and peptide D were combined and counted).

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Gary B. Nickol Ph.D. whose telephone number is 571-272-0835. The examiner can normally be reached on M-Th, 8:30-5:30; alternate Fri., 8:30-4:30.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Jeffrey Siew can be reached on 571-272-0787. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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Gary B. Nickol Ph.D.
Primary Examiner
Art Unit 1642

GBN



GARY NICKOL
PRIMARY EXAMINER